



PROTOCOL FOR TESTS ON DISTINCTNESS, UNIFORMITY AND STABILITY

Spinacia oleracea L.

SPINACH

UPOV Code: SPINA_OLE

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1. SUBJECT OF THE PROTOCOL AND REPORTING

1.1 Scope of the technical protocol

This Technical Protocol applies to all varieties of *Spinacia oleracea* L.

The protocol describes the technical procedures to be followed in order to meet the requirements of Council Regulation 2100/94 on Community Plant Variety Rights. The technical procedures have been agreed by the Administrative Council and are based on documents agreed by the International Union for the Protection of New Varieties of Plants (UPOV), such as the General Introduction to DUS (UPOV Document TG/1/3 http://www.upov.int/export/sites/upov/resource/en/tg_1_3.pdf), its associated TGP documents (<http://www.upov.int/tgp/en/>) and the relevant UPOV Test Guideline TG/55/7/ Rev.4 dated 16/03/2016 (http://www.upov.int/en/publications/tg-rom/tg055/tg_55_7.pdf) for the conduct of tests for Distinctness, Uniformity and Stability.

1.2 Entry into Force

The present protocol enters into force on **01.01.2017**. Any ongoing DUS examination of candidate varieties started before the aforesaid date will not be affected by the approval of the Technical Protocol. Technical examinations of candidate varieties are carried out according to the TP in force when the DUS test starts. The starting date of a DUS examination is considered to be the due date for submitting of plant material for the first test period.

In cases where the Office requests to take-over a DUS report for which the technical examination has either been finalized or which is in the process to be carried out at the moment of this request, such report can only be accepted if the technical examination has been carried out according to the CPVO TP which was in force at the moment when the technical examination started.

1.3 Reporting between Examination Office and CPVO and Liaison with Applicant

1.3.1 Reporting between Examination Office and CPVO

The Examination Office shall deliver to the CPVO a preliminary report ("the preliminary report") no later than two weeks after the date of the request for technical examination by the CPVO.

The Examination Office shall also deliver to the CPVO a report relating to each growing period ("the interim report") and, when the Examination Office considers the results of the technical examination to be adequate to evaluate the variety or the CPVO so requests, a report relating to the examination ("the final report").

The final report shall state the opinion of the Examination Office on the distinctness, uniformity and stability of the variety. Where it considers those criteria to be satisfied, or where the CPVO so requests, a description of the variety shall be added to the report. If a report is negative the Examination Office shall set out the detailed reasons for its findings.

The interim and the final reports shall be delivered to the CPVO as soon as possible and no later than on the deadlines as laid down in the designation agreement.

1.3.2 Informing on problems in the DUS test

If problems arise during the course of the test the CPVO should be informed immediately so that the information can be passed on to the applicant. Subject to prior permanent agreement, the applicant may be directly informed at the same time as the CPVO particularly if a visit to the trial is advisable.

1.3.3 Sample keeping in case of problems

If the technical examination has resulted in a negative report, the CPVO shall inform the Examination Office as soon as possible in case that a representative sample of any relevant testing material shall be kept.

2. MATERIAL REQUIRED

2.1 Plant material requirements

Information with respect to the agreed closing dates and submission requirements of plant material for the technical examination of varieties can be found on <http://cpvo.europa.eu/applications-and-examinations/technical-examinations/submission-of-plant-material-s2-publication> in the special issue S2 of the Official Gazette of the Office. General requirements on submission of samples are also to be found following the same link.

2.2 Informing the applicant of plant material requirements

The CPVO informs the applicant that

- he is responsible for ensuring compliance with any customs and plant health requirements.
- the plant material supplied should be visibly healthy, not lacking in vigour, nor affected by any important pest or disease.
- the plant material should not have undergone any treatment which would affect the expression of the characteristics of the variety, unless the competent authorities allow or request such treatment. If it has been treated, full details of the treatment must be given.

2.3 Informing about problems on the submission of material

The Examination Office shall report to the CPVO immediately in cases where the test material of the candidate variety has not arrived in time or in cases where the material submitted does not fulfil the conditions laid down in the request for material issued by the CPVO.

In cases where the examination office encounters difficulties to obtain plant material of reference varieties the CPVO should be informed.

3. METHOD OF EXAMINATION

3.1 Number of growing cycles

The minimum duration of tests should normally be two independent growing cycles.

The two independent growing cycles should be in the form of two separate plantings.

3.2 Testing Place

Tests are normally conducted at one place. In the case of tests conducted at more than one place, guidance is provided in TGP/9 "Examining Distinctness" http://www.upov.int/edocs/tgpdocs/en/tgp_9.pdf.

3.3 Conditions for Conducting the Examination

The tests should be carried out under conditions ensuring satisfactory growth for the expression of the relevant characteristics of the variety and for the conduct of the examination.

3.4 Test design

3.4.1 Each test should be designed to result in a total of at least 100 single spaced plants which should be divided between at least two replicates.

3.4.2 The design of the tests should be such that plants or parts of plants may be removed for measurement or counting without prejudice to the observations which must be made up to the end of the growing cycle.

3.5 Additional tests

In accordance with Article 83(3) of Council Regulation No. 2100/94 an applicant may claim either in the Technical Questionnaire or during the test that a candidate has a characteristic which would be helpful in establishing distinctness. If such a claim is made and is supported by reliable technical data, an additional test may be undertaken providing that a technically acceptable test procedure can be devised.

Additional tests will be undertaken, with the agreement of the President of CPVO, where distinctness is unlikely to be shown using the characters listed in the protocol.

3.6 Constitution and maintenance of a variety collection

The process for the constitution and the maintenance of a variety collection can be summarized as follows:

Step 1: Making an inventory of the varieties of common knowledge.

Step 2: Establishing a collection ("variety collection") of varieties of common knowledge which are relevant for the examination of distinctness of candidate varieties.

Step 3: Selecting the varieties from the variety collection which need to be included in the growing trial or other tests for the examination of distinctness of a particular candidate variety.

3.6.1 Forms of variety collection

The variety collection shall comprise variety descriptions and living plant material, thus a living reference collection. The variety description shall be produced by the EO unless special cooperation exists between EOs and the CPVO. The descriptive and pictorial information produced by the EO shall be held and maintained in a form of a database.

3.6.2 Living Plant Material

The EO shall collect and maintain living plant material of varieties of the species concerned in the variety collection.

3.6.3 Range of the variety collection

The living variety collection shall cover at least those varieties that are suitable to climatic conditions of a respective EO.

3.6.4 Making an inventory of varieties of common knowledge for inclusion in the variety collection

The inventory shall take into account the list of protected varieties and the official, or other, registers of varieties, in particular:

The inventory shall include varieties protected under National PBR (UPOV contracting parties) and Community PBR, varieties registered in the Common Catalogue, the OECD list, the Conservation variety list and varieties in trade or in commercial registers for those species not covered by a National or the Common Catalogue.

3.6.5 Maintenance and renewal/update of a living variety collection

The EO shall maintain seeds in conditions which will ensure germination and viability, periodical checks, and renewal as required. For the renewal of existing living material the identity of replacement living plant material shall be verified by conducting side-by-side plot comparisons between the material in the collection and the new material.

4. ASSESSMENT OF DISTINCTNESS, UNIFORMITY AND STABILITY

The prescribed procedure is to assess distinctness, uniformity and stability in a growing trial.

4.1 Distinctness

4.1.1 General recommendations

It is of particular importance for users of this Technical Protocol to consult the UPOV-General Introduction to DUS (link in chapter 1 of this document) and TGP 9 'Examining Distinctness' (http://www.upov.int/edocs/tgpdocs/en/tgp_9.pdf) prior to making decisions regarding distinctness. However, the following points are provided for elaboration or emphasis in this Technical Protocol.

Further guidance is provided in documents TGP/9 "Examining Distinctness" and TGP/8 "Trial Design and Techniques Used in the Examination of Distinctness, Uniformity and Stability".

4.1.2 Consistent differences

The differences observed between varieties may be so clear that more than one growing cycle is not necessary. In addition, in some circumstances, the influence of the environment is not such that more than a single growing cycle is required to provide assurance that the differences observed between varieties are sufficiently consistent. One means of ensuring that a difference in a characteristic, observed in a growing trial, is sufficiently consistent is to examine the characteristic in at least two independent growing cycles.

4.1.3 Clear differences

Determining whether a difference between two varieties is clear depends on many factors, and should consider, in particular, the type of expression of the characteristic being examined, i.e. whether it is expressed in a qualitative, quantitative, or pseudo-qualitative manner. Therefore, it is important that users of these Technical Protocols are familiar with the recommendations contained in the UPOV-General Introduction to DUS prior to making decisions regarding distinctness.

Decision standards

4.1.4 Number of plants/parts of plants to be examined

Unless otherwise indicated, for the purposes of distinctness, all observations on single plants should be made on 60 plants or parts taken from each of 60 plants and any other observations made on all plants in the test, disregarding any off-type plants.

4.1.5 Method of observation

The recommended method of observing the characteristic for the purposes of distinctness is indicated by the following key in the third column of the Table of Characteristics (see document TGP/9 "Examining Distinctness", Section 4 "Observation of characteristics"):

MG:	single measurement of a group of plants or parts of plants
MS:	measurement of a number of individual plants or parts of plants
VG:	visual assessment by a single observation of a group of plants or parts of plants
VS:	visual assessment by observation of individual plants or parts of plants

Type of observation: visual (V) or measurement (M)

"Visual" observation (V) is an observation made on the basis of the expert's judgment. For the purposes of this document, "visual" observation refers to the sensory observations of the experts and, therefore, also includes smell, taste and touch. Visual observation includes observations where the expert uses reference points (e.g. diagrams, example varieties, side-by-side comparison) or non-linear charts (e.g. colour charts). Measurement (M) is an objective observation against a calibrated, linear scale e.g. using a ruler, weighing scales, colorimeter, dates, counts, etc.

Type of record: for a group of plants (G) or for single, individual plants (S)

For the purposes of distinctness, observations may be recorded as a single record for a group of plants or parts of plants (G), or may be recorded as records for a number of single, individual plants or parts of plants (S). In most cases, "G" provides a single record per variety and it is not possible or necessary to apply statistical methods in a plant-by-plant analysis for the assessment of distinctness.

In cases where more than one method of observing the characteristic is indicated in the Table of Characteristics (e.g. VG/MG), guidance on selecting an appropriate method is provided in document TGP/9, Section 4.2.

4.2 Uniformity

It is of particular importance for users of this Technical Protocol to consult the UPOV-General Introduction to DUS (link in chapter 1 of this document) and TGP 10 'Examining Uniformity' (http://www.upov.int/edocs/tgpdocs/en/tgp_10.pdf) prior to making decisions regarding uniformity. However, the following points are provided for elaboration or emphasis in this Technical Protocol:

For the assessment of uniformity of hybrid varieties, a population standard of 2% and an acceptance probability of at least 95% should be applied to off-types excluding clearly recognisable inbred plants. In the case of a sample size of 100 spaced plants, 5 off-types are allowed. In addition, a population standard of 3% with the same acceptance probability should be applied to clearly recognisable inbred plants. In the case of a sample size of 100 spaced plants the additional number of clearly recognisable inbred plants would be 6.

4.3 Stability

4.3.1 It is of particular importance for users of this Technical Protocol to consult the UPOV-General Introduction to DUS (link in chapter 1 of this document) and TGP 11 'Examining Stability' (http://www.upov.int/edocs/tgpdocs/en/tgp_11.pdf).

In practice, it is not usual to perform tests of stability that produce results as certain as those of the testing of distinctness and uniformity. However, experience has demonstrated that, for many types of variety, when a variety has been shown to be uniform, it can also be considered to be stable.

4.3.2 Where appropriate, or in cases of doubt, stability may be further examined by testing a new seed stock to ensure that it exhibits the same characteristics as those shown by the initial material supplied.

5. GROUPING OF VARIETIES AND ORGANIZATION OF THE GROWING TRIAL

- 5.1** The selection of varieties of common knowledge to be grown in the trial with the candidate varieties and the way in which these varieties are divided into groups to facilitate the assessment of distinctness are aided by the use of grouping characteristics.
- 5.2** Grouping characteristics are those in which the documented states of expression, even where produced at different locations, can be used, either individually or in combination with other such characteristics: (a) to select varieties of common knowledge that can be excluded from the growing trial used for examination of distinctness; and (b) to organize the growing trial so that similar varieties are grouped together.
- 5.3** The following have been agreed as useful grouping characteristics.
- a) Plant: red coloration of petioles and veins (characteristic 3)
 - b) Leaf blade: intensity of green colour (characteristic 4)
 - c) Leaf blade: blistering (characteristic 5)
 - d) Proportion of monoecious plants (characteristic 14)
 - e) Proportion of female plants (characteristic 15)
 - f) Proportion of male plants (characteristic 16)
 - g) Time of start of bolting (for spring sown crops, 15% of plants) (characteristic 17)
 - h) Resistance to *Peronospora farinosa* f. sp. *spinaciae* race Pfs:10 (characteristic 19.9)
 - i) Resistance to *Peronospora farinosa* f. sp. *spinaciae* race Pfs:12 (characteristic 19.11)
 - j) Resistance to *Peronospora farinosa* f. sp. *spinaciae* race Pfs:13 (characteristic 19.12)
- 5.4** If other characteristics than those from the TP are used for the selection of varieties to be included into the growing trial, the EO shall inform the CPVO and seek the prior consent of the CPVO before using these characteristics.

6. INTRODUCTION TO THE TABLE OF CHARACTERISTICS

6.1 Characteristics to be used

The characteristics to be used in DUS tests and preparation of descriptions shall be those referred to in the table of characteristics. All the characteristics shall be used, providing that observation of a characteristic is not rendered impossible by the expression of any other characteristic, or the expression of a characteristic is prevented by the environmental conditions under which the test is conducted or by specific legislation on plant health. In the latter case, the CPVO should be informed.

The Administrative Council empowers the President, in accordance with Article 23 of Commission Regulation N°874/2009, to insert additional characteristics and their expressions in respect of a variety.

Technical Protocols with asterisked characteristics (only for certain vegetable species)

In the case of disease resistance characteristics, only those resistances marked with an asterisk (*) in the CPVO column are compulsory.

In accordance to the approval by the CPVO Administrative Council on 01/10/2015 of the procedure "The use of disease resistance characteristics in CPVO vegetable Technical Protocols" (DOC-AC-2015-2-18), a phasing-in period is established for the new asterisked diseases resistance characteristics set out in the present protocol TP-55/5 Rev. The phasing-in period for TP-55/5 Rev. has been established for three years, and will cease to apply on 01/01/2019, at which time the characteristics in question will become obligatory.

The characteristics in question are the following:

- (*) 19.9: Resistance to *Peronospora farinosa* f. sp. *spinaciae* race Pfs:10
- (*) 19.11: Resistance to *Peronospora farinosa* f. sp. *spinaciae* race Pfs:12
- (*) 19.12: Resistance to *Peronospora farinosa* f. sp. *spinaciae* race Pfs:13

States of expression and corresponding notes

In the case of qualitative and pseudo-qualitative characteristics, all relevant states of expression are presented in the characteristic. However, in the case of quantitative characteristics with 5 or more states, an abbreviated scale may be used to minimize the size of the Table of Characteristics. For example, in the case of a quantitative characteristic with 9 states, the presentation of states of expression in the Test Guidelines may be abbreviated as follows:

State	Note
small	3
medium	5
large	7

However, it should be noted that all of the following 9 states of expression exist to describe varieties and should be used as appropriate:

State	Note
very small	1
very small to small	2
small	3
small to medium	4
medium	5
medium to large	6
large	7
large to very large	8
very large	9

6.2 Example Varieties

Where appropriate, example varieties are provided to clarify the states of expression of each characteristic.

6.3 Legend

G	Grouping characteristic	– see Chapter 5
(*)	Asterisked characteristic	– see Chapter 6.1.2
MG, MS, VG, VS	– see Chapter 4.1.5	
QL	Qualitative characteristic	
QN	Quantitative characteristic	
PQ	Pseudo-qualitative characteristic	

Legend: Explanations covering several characteristics

- (a) See Explanations on the Table of Characteristics in Chapter 8.1
- (+) See Explanations on the Table of Characteristics in Chapter 8.

7. TABLE OF CHARACTERISTICS

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
1.		VG	Seed: spines		
			absent	Butterflay, Marimba	1
QL			present	Breedblad, Scherpzaad	9
2.	1.	VG	Seedling: length of cotyledon		
			short	Nores	3
			medium		5
QN			long	Breedblad Scherpzaad, Resistoflay	7
3.	2. (*)	VG	Plant: red coloration of stem, petioles and veins		
			absent	Resistoflay, Nores	1
(+)			present	Red Cardinal, Reddy	9
QL G					
4.	3. (*)	VG (a)	Leaf blade: intensity of green colour		
			very light		1
			light	Monet, Viroflay, Winterreuzen	3
			medium	Butterflay, Monnopa	5
			dark	Imola, Lavewa, Nores	7
G			very dark	Lorelay, Mystic	9
5.	4. (*)	VG (a)	Leaf blade: blistering		
			absent or very weak	Matador	1
			weak	Polka, Tarpv	3
			medium	Butterflay, Koala, Mystic	5
			strong	Giraffe, Rhythm	7
G			very strong	Menorca, Revolver	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note		
6.	5.	VG	Leaf blade: lobing				
			(*)	(a)	absent or very weak	1	
			(+)		weak	Butterflay, Giraffe	3
			QN		medium	Mystic	5
			strong	Parrot	7		
7.	6.	VG	Petiole: attitude				
			(*)	(a)	erect	Grappa	1
			(+)		semi-erect	Monnopa, Parrot	3
QN		horizontal	Comte, Lavewa	5			
8.	7.	VG	Petiole: length				
			(a)	short	Imola, Mystic	3	
				medium	Butterflay, Giraffe	5	
			long	Grappa, Resistoflay	7		
9.	8.	VG	Leaf blade: attitude				
			(*)	(a)	erect	1	
			(+)		semi-erect	Grappa, Monnopa, Rhythm	3
			QN		horizontal	Lavewa, Mystic	5
			semi-pendulous	Giraffe, Medania	7		
10.	9.	VG	Leaf blade: shape (excluding basal lobes)				
			(*)	(a)	triangular	Grappa, Maracas	1
			PQ		medium ovate	Lavewa, Resistoflay	2
					broad ovate	Butterflay	3
					medium elliptic		4
					broad elliptic	Nores	5
		circular	Giraffe	6			

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
11. QN	10.	VG (a)	Leaf blade: curving of margin		
			incurved		1
			flat	Resistoflay	2
			recurved	Imola	3
12. QN	11. (*)	VG (a)	Leaf blade: shape of apex		
			acute	Grappa, Rhythm	1
			obtuse	Resistoflay	2
			rounded	Imola, Nores	3
13. QN	12. (*)	VG (a)	Leaf blade: shape in longitudinal section		
			concave		1
			flat	Mystic, Resistoflay	2
			convex	Grappa, Lazio	3
14. (+) QN G	13. (*)	VS	Proportion of monoecious plants		
			absent or very low	Medania	1
			low	Matador	3
			medium	Figo	5
			high	Giraffe, Lazio	7
			very high	Monnopa	9
15. (+) QN G	14. (*)	VS	Proportion of female plants		
			absent or very low	Monnopa	1
			low	Giraffe	3
			medium	Figo, Medania	5
			high	Parrot	7
			very high		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note	
16.	15.	VS	Proportion of male plants			
			(*)	absent or very low	Monnopa, Parrot	1
			(+)	low		3
			QN	medium	Medania	5
				high		7
G		very high		9		
17.	16.	MG	Time of start of bolting (for spring sown crops, 15% of plants)			
			(*)	very early	Figo, Maracas	1
			(+)	early	Bandola, Viroflay	3
			QN	medium	Matador, Monnopa	5
				late	Grappa, Medania, Revolver	7
G		very late	Chica, Lavewa	9		
18.	17.	VG	Seed: spines			
			(+)	absent	Resistoflay	1
QL		present	Breedblad Scherpzaad, Marimba	9		
19.	18.	VS/VG	Resistance to <i>Peronospora farinosa</i> f. sp. <i>spinaciae</i>			
(+)						
QL						
19.1	18.1		Race Pfs:1			
			absent	Viroflay, Winterreuzen	1	
			present	Califlay, Resistoflay	9	
19.2	18.2		Race Pfs:2			
			absent	Califlay	1	
			present	Resistoflay	9	
19.3	18.3		Race Pfs:3			
			absent	Resistoflay	1	
			present	Califlay, Clermont	9	

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
19.4	18.4		Race Pfs:4		
			absent	Califlay	1
			present	Clermont	9
<hr/>					
19.5	18.5		Race Pfs:5		
			absent	Clermont	1
G			present	Califlay, Campania	9
<hr/>					
19.6	18.6		Race Pfs:6		
			absent	Califlay, Campania	1
G			present	Boeing	9
<hr/>					
19.7	18.7		Race Pfs:7		
			absent	Califlay	1
G			present	Campania	9
<hr/>					
19.8	18.8		Race Pfs:8		
			absent	Boeing, Campania	1
			present	Lazio, Lion	9
<hr/>					
19.9 (*)	18.9		Race Pfs:10		
			absent	Boeing, Campania, Lion	1
			present	Lazio	9
<hr/>					
19.10	18.10		Race Pfs:11		
			absent	Lazio	1
			present	Boeing, Campania, Lion, Whale	9
<hr/>					
19.11 (*)	18.11		Race Pfs:12		
			absent	Boeing, Campania	1
			present	Finch, Pigeon, Red Kitten, Zebu	9
<hr/>					
19.12 (*)	18.12		Race Pfs:13		
			absent	Campania	1
			present	Boeing, Lion	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
19.13	18.13		Race Pfs:14		
QL			absent	Campania, Pigeon	1
			present	Califray, Lion	9
19.14	18.14		Race Pfs:15		
QL			absent	Caladonia	1
			present	Pigeon	9
19.15			Race Pfs:16		
QL			absent	Meerkat	1
			present	Caladonia	9
20.	18.	VG	Resistance to Cucumber mosaic virus (CMV)		
(+)			absent	Polka	1
QL			present	Symphony	9

8. EXPLANATIONS ON THE TABLE OF CHARACTERISTICS

8.1 Explanations covering several characteristics

Characteristics containing the following key in the third column of the Table of Characteristics should be examined as indicated below

(a) Observations on the leaf blade should be made on the seventh to tenth leaves of the adult not bolted plant. The shape of the leaf blade in longitudinal section should be observed on central leaves.

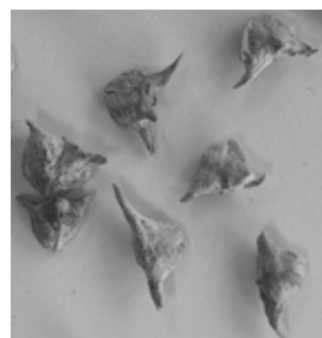
8.2 Explanations for individual characteristics

Ad 1: Seed: spines

To be observed on submitted seed



1
absent



9
present

Ad 3: Plant: red coloration of stem, petioles and veins



1
absent

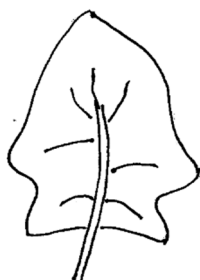


9
present

Ad 6: Leaf blade: lobing



1
absent or very weak



3
weak



5
medium



7
strong

Ad 7: Petiole: attitude



1
erect



3
semi-erect



5
horizontal

Ad 9: leaf blade: attitude

To be observed in relation to the horizontal, independent of the attitude of the petiole (characteristic 5).



1
erect



3
semi-erect



5
horizontal



7
semi-pendulous

Ad 14: Proportion of monoecious plants

Ad 15: Proportion of female plants

Ad 16: Proportion of male plants

Observations on the proportion of monoecious, female or male plants should be made at the beginning of seed setting. The three groups are defined as follows:

Monoecious plants: plants which have both male flowers and female flowers (seeds clearly visible);
Female plants: plants which have only female flowers (seeds clearly visible);
Male plants: plants which have only male flowers.

	<u>Note</u>	<u>Approximate percentage</u>
absent or very low	1	< 10%
	2	20%
low	3	30%
	4	40%
medium	5	50%
	6	60%
high	7	70%
	8	80%
very high	9	> 90%

Ad 17: Time of start of bolting (for spring sown crops, 15% of plants)

The time of bolting of a plant is when the central flowering stem appears through stretching of the internodes.

Ad 18: Seed: spines

To be observed on harvested seed



1
absent



9
present

Ad 19: Resistance to *Peronospora farinosa* f. sp. *spinaciae*

Maintenance of races

Type of medium: Living host plants, obtainable from:
Naktuinbouw
P.O. Box 40
NL-2370 AA Roelofarendsveen
Netherlands
www.naktuinbouw.com
or plant material with spores stored at -20° C for a maximum of one year

Execution of test:

Growth stage of plants:	First cotyledons/leaf, eleven-day-old plants
Temperature:	15°C during day/12°C during night
Light:	15 hours per day, after emergence
Growing method:	In soil in pots or trays in a glasshouse or growth chamber
Method of inoculation:	Sporulating leaves, taken from host plants that were infected seven days before, are thoroughly rinsed with sterile tap water (maximum 150 ml water per 224 plants). The spore suspension is filtered through cheesecloth and sprayed on test plants until the inoculum covers the leaves but does not run off. 150 ml of suspension is enough for up to 3 x 224 plants. Spore density should be 20,000 to 100,000 conidia/ml water. The spore suspension should be used fresh.
Remarks:	<p>Spinach downy mildew is wind-borne. Sporulating plants should be kept in closed containers or isolated chambers to prevent any cross-contamination. Resistant controls are needed in each multiplication and in each test to ensure the race identity.</p> <p>Light and humidity conditions during seedling development and incubation are critical. Optimal humidity of approximately 80-90% RH allows plant growth and fungal growth; strong light inhibits spore germination and infection.</p> <p>The test should be carried out in wintertime with protection against direct sunshine. After inoculation, the plants should remain under plastic for three days. After this time, the plastic should be slightly raised during the daytime.</p>

Duration of test:

- Multiplication	harvest spores 7 days after inoculation
- Sowing to inoculation:	11 days
- Inoculation to reading:	10 days

Number of plants tested: at least 20 plants

Evaluation of infection: Resistance is usually complete; sometimes necrotic spots are visible as a result of infection. Susceptible plants show varying degrees of sporulation. Sporulation is visible as a grey covering on leaves, starting on the more humid abaxial side.

Differential varieties to identify races:

Races Pfs:1-8 and 10-16 of *Peronospora farinosa* f. sp. *spinaciae* are defined with a standard set of "differential varieties" according to the following table:

Differential variety	Pfs:1	Pfs:2	Pfs:3	Pfs:4	Pfs:5	Pfs:6	Pfs:7	Pfs:8	Pfs:10	Pfs:11	Pfs:12	Pfs:13	Pfs:14	Pfs:15	Pfs:16
Vioflay	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Resistoflay	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S
Califlay	R	S	R	S	R	S	S	R	S	R	R	S	R	S	R
Clermont	R	R	R	R	S	S	S	S	S	S	S	S	S	R	S
Campania	R	R	R	R	R	S	R	S	S	R	S	S	S	R	R
Boeing	R	R	R	R	R	R	R	S	S	R	S	R	S	R	R
Lion	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R
Lazio	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S
Whale	R	R	R	R	R	R	R	R	S	R	R	S	R	S	R
Pigeon	R	R	R	R	R	R	R	R	R	R	R	R	S	R	S
Caladonia	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R
Meerkat	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S

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Legend: R= resistance present; S = resistance absent, susceptible

Ad 20: Resistance to Cucumber mosaic virus (CMV)

Maintenance and propagation of isolates

Storage of medium: on leaves in freezer or desiccated over CaCl₂

Special conditions: Isolates NL 16 and SP 43 which can be obtained from:
PRI (Plant Research International)
Prime Diagnostics
P.O. Box 16
NL-6700 AA Wageningen
Netherlands
www.primediagnosics.nl

Propagation: on susceptible cucumber plants

Execution of test

Growth stage of plants: when two or three true leaves are present

Temperature: 20°C during the day, 18°C during the night

Light: at least 16 hours per day

Growing method: plants grown in 5 x 5 cm module (potting soil)

Preparation of inoculum: a mixture of isolates is ground in water (dilution 1:10)

Method of inoculation: plants are dusted with carborundum powder on two or three leaves and then rubbed with a sponge soaked in inoculum. After inoculation, the plants are lightly rinsed with water.

Remarks: due to climatic conditions, the test is best carried out from February to June (Northern Hemisphere).

Observations

Time of observation: 7 to 9 days after inoculation

Symptoms:

resistant plant: no symptoms

sensitive plant: dwarf growth, mosaic symptoms in the heart of the plants

Differential host varieties to be used:

susceptible variety: Polka

resistant variety: Symphony

9. LITERATURE

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10. TECHNICAL QUESTIONNAIRE

The Technical Questionnaire is available on the CPVO website under the following reference:
CPVO-TQ/055/5 Rev.2